PATENT
Attorney Docket No. 62611.000202

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	:	MORSEMAN, et al.	Confirmation No.:	6731
Application No.:	:	09/882,376		
Filed	:	June 18, 2001		
Title	:	HIGH FLUORESCENT INTENSITY CROSS-LINKED ALLOPHYCOCYANIN		
TC/Art Unit	:	1641		
Examiner:	:	Counts, Gary W.		
Docket No.	:	62611.000202		
Customer No.	:	**21967**		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir,

I, Mark Wesley Moss, declare that:

1.) I attended The Johns Hopkins University from 1988 through 1992, in pursuit of a Bachelor of Science degree in Chemical Engineering.

2.) I have been employed by Martek Biosciences Corporation ("Martek") since 1997, where I have performed research in the field of immunoassay design and flow cytometry, and have co-authored several peer reviewed publications relating to the use of algal phycobiliproteins in various immunoassay applications, including flow cytometry. A recitation of some of these publications, together with details of my education, are given in the short version of my curriculum vitae, which is attached as **Exhibit A**.

3). While employed at Martek, I have developed phycobiliprotein-based reagents that are currently commercialized for use in applications such as flow cytometry and protein microarrays. Representative experiments using both techniques are described below; and these experiments as described were performed or supervised by me.

4.) While employed at Martek, I participated in the development of a new method for stabilizing allophycocyanin (APC) which avoided the use of strongly chaotropic agents during cross-linking and recovery of APC. The resultant cross-linked APC which had not been exposed to strongly chaotropic agents (hereinafter "Applicant's cross-linked APC") was offered for sale to a third party, at a fixed price, on or about December 22, 1998. Applicants' product was represented and sold as a preparation containing cross-linked allophycocyanin. To the best of my knowledge, the third party to whom Applicants offered to sell Applicants' product did not use Applicants' product more than one year prior to the filing date of the '376 application for a use that involved time-resolved fluorescence.

5.) Therefore, Applicants did not offer to sell Applicants' product on or about December 22, 1998 to any third party who, to the best of Applicants' knowledge, used Applicants' product in time-resolved fluorescence assays more than one year prior to the filing date of the '376 application.

6.) Subsequently, it was discovered that use of Applicant's cross-linked APC in time-resolved fluorescence assays conferred enhanced performance compared to conventional cross-linked APC, and the patent application designated by U.S. Serial No. 09/882,376 was prepared and filed. I am a named inventor of U.S. Patent Application Serial Number 09/882,376 (the "'376 application").

7.) I have read, and am familiar with, the following documents:

a.) The '376 application;

b.) The Park, et al., publication;

c.) My Declaration submitted in the '376 application on March 1, 2005; and

d.) The Office Action issued by the USPTO in the '376 application on March 29, 2005 (the "March 29, 2005 Office Action").

8.) I understand that claims 3-14 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Park et al., Homogenous Proximity Tyrosine Kinase Assays, Anal. Biochem. 269: 94-104 (1999) (the "Park, et al., publication") in view of the Statement by Applicant dated December 3, 2003 ("Applicants' Statement") relating to Applicants' offer to

sell cross-linked allophycocyanin which had not been exposed to strongly chaotropic agents ("Applicants' product").

9.) Claim 3 of the '376 application is drawn to improved fluorescent assay methods where the methods involve detecting fluorescence of allophycocyanin (APC) in a time-resolved manner and the improvement involves using labels comprising cross-linked APC that has not been exposed to strongly chaotropic agents ("Applicant's cross-linked APC").

10.) I understand that, during prosecution of the '376 application, the Examiner has taken the position that the Park, et al., publication teaches all elements of claim 3 of the '376 application, except the use of cross-linked APC that has not been exposed to strongly chaotropic agents. It has been acknowledged that cross-linked APC that has not been exposed to strongly chaotropic agents (Applicant's cross-linked APC) was made "available for sale by Applicant more than one year prior to the filing date of [the '376 application]." The Examiner has asserted that "a skilled artisan would have a reasonable expectation" that "both [the conventional cross-linked APC and Applicant's cross-linked APC] would perform equally well in Time Resolved Fluorescence Assays."

11.) Based on my experience in the field of fluorescent specific binding assays, I have been asked to comment on whether the results of the Flow Cytometric Analysis **[Exhibit B]** and of the Immunoassay Analysis **[Exhibit C]** demonstrate that Applicants' product performs equally well compared with conventional APC in standard enzyme binding assays, or if Applicant's cross-linked APC produces improved results over conventional cross-linked APC.

12.) The Molecular Probes'™ XL-APC is cross-linked allophycocyanin prepared by the use of a strongly chaotropic agent ("conventional APC"), as described in Product Information distributed by Molecular Probes, attached to my Declaration of March 1, 2005, and attached hereto as Exhibit D. The cross-linked APC described in Park, et al., corresponds to this conventional APC. XL-APC conjugated to streptavidin is used for numerous procedures including flow cytometry and indirect immunoassays. *See* Molecular Probes' Product Information, page 3, column 1 **[Exhibit D]**. These procedures do not involve time-resolved fluorescence.

13.) Routine Flow Cytometric Analysis involves detection of steady-state fluorescence from labeled cells. Cross-linked APC is well known as one of the label compounds. Exhibit B shows the comparison of prior art cross-linked APC to SL-APC, as measured in a commercial Fluorescence Activated Cell Sorting (FACS) apparatus. The flow cytometry used in the Flow Cytometric Analysis was a standard binding assay, in which fluorescence of bound labels was measured without any time-dependent gating. Thus, the Flow Cytometric Analysis did not involve time-resolved fluorescence. The Molecular Probes'™ XL-APC used in the Flow Cytometric Analysis was cross-linked allophycocyanin prepared by the use of a strongly chaotropic agent ("conventional APC").

14.) This experiment involved labeling mouse EL4 cells with 0.5 µg of biotinylated anti-mouse Thy1.2 antibody in 100 µl of PBS, pH 7.4, with 1% BSA, washing in PBS/BSA, and then staining the cells with 4 µg of either (a) Molecular Probes'™ XL-APC conjugated with streptavidin in 100 µl of the same solution or (b) Applicants' product conjugated with streptavidin in 100 µl of the same solution. After washing again in the PBS/BSA, the cells were analyzed on a FACSVantage™ SE flow cytometer with a 633 HeNe laser and a 670 nm emission filter.

15.) The results of the Flow Cytometric Analysis show that Applicants' product produced a mean channel fluorescence that was slightly less than the mean channel fluorescence of Molecular Probes'™ XL-APC. Molecular Probes'™ XL-APC generated a mean channel fluorescence of 1328.4, while Applicants' product generated a mean channel fluorescence of 1158.5. Thus, Applicants' product demonstrated a similar (but slightly lower) fluorescence intensity measurement than Molecular Probes'™ XL-APC in the Flow Cytometric Analysis. **[Exhibit B]**.

16.) Consequently, based on my experience and as further evidenced by the Flow Cytometric Analysis in Exhibit B, Applicants' product does not produce improved results over conventional APC in steady-state fluorescence binding assays such as flow cytometry.

17.) Microplate readers for fluorescence detection of antibody binding also detect steady-state fluorescence. The Immunoassay Analysis shown in Exhibit C was a standard binding assay, in which fluorescence of bound labels was measured without any time-

dependent gating. The Immunoassay Analysis, therefore, did not involve time-resolved fluorescence.

18.) The experiment shown in Exhibit C involved a black polystyrene microplate coated with mouse IgG in 100 mM sodium carbonate, pH 9.6, blocked with 100 mM sodium phosphate, pH 7.4, with 150 mM NaCl and 1% BSA, and then incubated with serial dilutions of biotinylated goat-anti-mouse IgG for one (1) hour at 37°C. The plate was washed with PBS with 0.1% Tween 20 and then incubated for one (1) hour at room temperature with either Applicants' product conjugated with streptavidin or Molecular Probes'™ XL-APC conjugated with streptavidin, both at 10 ug/ml in 100 mM sodium phosphate, pH 7.4, with 150 mM NaCl. The plate was then washed with the PBS-Tween again and read on a Wallac Victor™ Multi-Label Reader using excitation at 590 nm and emission at 660 nm. The Molecular Probes'™ XL-APC used in the Immunoassay Analysis was conventional APC.

19.) The results of the Immunoassay Analysis show that the overall intensity of signal obtained by SL-APC was not significantly increased over that of the Molecular Probes™ XL-APC. The insignificant differences in $LogEC_{50}$ values from the dose response curves may be explained by minor differences in final streptavidin-conjugation ratios between the two reagents. . Also, the Molecular Probes'™ XL-APC achieved slightly higher fluorescence intensity measurement at some points on the dose response curve, displaying a fluorescence of approximately 19000 cps at a biotinylated goat-anti-mouse IgG concentration of log -5.5 Molar, compared to approximately 17000 counts for SL-APC. Applicants' product, therefore, did not produce a fluorescence intensity measurement significantly higher than Molecular Probes'™ XL-APC in the Immunoassay Analysis. **[Exhibit C]**.

20.) Accordingly, based on my experience and as further evidenced by the Immunoassay Analysis, Applicants' product does not produce improved results over conventional APC in steady-state solid phase antibody binding assays.

21.) In contrast, Examples 6 and 8 of the '376 application show the results of experiments comparing Applicant's cross-linked APC (GL-APC and SL-APC) to conventional APC (XL-APC) in time resolved fluorescence assays, and both Examples show significantly improved performance using Applicant's cross-linked APC in signal-to-noise ratio and signal intensity.

22) In Example 6, SL-APC provides an increase in signal-to-noise ratios over XL-APC when measuring phosphorylated poly-GAT ranging from 31.8% to 65.6% in a 96 well plate, and from 18.1% to 36.1% in a 384 well plate (See Table 3, pp.15-16). Figure 5 also illustrates the increase in ratiometric signal intensity over XL-APC.

23) Example 8 shows that in a non-receptor *src* tyrosine kinase assay, SL-APC demonstrated a 37.6% increase in signal-to-noise ratio, and a 100.6% increase in signal intensity over XL-APC (Table 4, p. 20). The difference in signal intensity and signal-to-noise between SL-APC and XL-APC for the insulin receptor kinase assay was not as large, but the SL-APC still showed a slight increase.

24) The data from these examples shows that when used on an equal mass per test basis, SL-APC outperforms XL-APC in intensity and signal-to-noise ratios, and that it can still match the XL-APC results even when used at a lower concentration. This provides the end user the means to obtain more optimal assay results compared to XL-APC using the same amount of reagent per test, or similar assay results using less reagent per test, with either case constituting an advantage to the user. Thus, Applicant's cross-linked APC performs surprisingly better than conventional cross-linked APC specifically in time-resolved fluorescence assays.

25.) The undersigned acknowledges that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon. The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Executed on: 9-27-05

Declarant's Signature: ______________________
Mark W. Moss

CURRICULUM VITAE
Mark Wesley Moss

Current Position

Martek Biosciences, Columbia, MD- Group Leader, Research and Development, Detection Systems Group

Professional Background

2002-04 **Martek Biosciences,** Columbia, MD- Research Associate III, Research and Development, Detection Systems Group

1997-97 **Martek Biosciences**, Columbia, MD- Research Associate II, Fluorescent Products Group

1994-97 **The Johns Hopkins University School of Medicine**, Division of Infectious Disease, Baltimore, MD- Senior Laboratory Technician II, International HIV/AIDS Reference Laboratory, NIH/NIAID

1990-94 **The Johns Hopkins University School of Medicine**, Division of Infectious Disease, Baltimore, MD- Laboratory Technician, International HIV/AIDS Reference Laboratory, NIH/NIAID

Education

1988-92 **The Johns Hopkins University**, Baltimore MD. – BS Program, Chemical Engineering

Societies

The Society for Biomolecular Screening
The International Society for Analytical Cytology
The Chesapeake Cytometry Consortium

Professional Experience

Laboratory

Protein microarray design and optimization
Flow cytometry, including operation and maintenance of in-house fluorescent cell sorter
Isolation, derivitization, and bioconjugation of fluorescent proteins and other biomolecules
Fluorescent, chemifluorescent, and enzyme-linked immunoassays and assay development
UV/Vis absorbance and fluorescence emission spectroscopy
Aqueous and organic chemistry methods
Western blotting
Mammalian cell culture
Column chromatography
PCR and RT-PCR

Business development

Intellectual property development
Tradeshows and conferences
Conversion of data and technical works into marketing materials
Graphics for tradeshows and marketing pieces

Other Experience

Biosafety level 3 training and work experience
Yearly OSHA Hazards Communications training

Publications

Telford WG. Moss MW. Morseman JP. Allnutt FCT. Cyanobacterial Stabilized Phycobilisomes as Fluorochromes for Extracellular Antigen Detection by Flow Cytometry. **JOURNAL OF IMMUNOLOGICAL METHODS. 254(1-2): 13-30, 2001 Aug 1.**

Telford WG. Moss MW. Morseman JP. Allnutt FCT. Cryptomonad Algal Phycobiliproteins as Fluorochromes for Extracellular and Intracellular Antigen Detection by Flow Cytometry. **CYTOMETRY. 44(1): 16-23, 2001 May 1.**

Zoha SJ. Ramnarain S. Morseman JP. Moss MW. Allnutt FCT. Rogers Y. Harvey B. PBXL Fluorescent Dyes for Ultrasensitive Direct Detection. **JOURNAL OF FLUORESCENCE. 9(3):197-208, 1999 Oct.**

Morseman JP, Moss MW, Zoha SJ, Allnutt FC. PBXL-1: a new fluorochrome applied to detection of proteins on membranes. **BIOTECHNIQUES. 26:559-63, 1999 Mar.**

Moss MW. Carella AV. Provost V. Quinn TC. Comparison of absolute $CD4^+$ lymphocyte counts by enzyme immunoassay(TRAx CD4 Test Kit) and flow cytometry. **CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY. 3(4):371-3, 1996 Jul.**

Carella AV. Moss MW. Provost V. Quinn TC. A manual bead assay for the determination of absolute $CD4^+$ and $CD8^+$ counts in human immunodeficiency virus-infected individuals. **CLINICAL AND DIAGNOSITIC LABORATORY IMMUNOLOGY. 2(5):623-5, 1995 Sep.**

Asihene PJ. Kline RL. Moss MW. Carella AV. Quinn TC. Evaluation of rapid test for detection of antibody to human immunodeficiency virus type 1 and type 2. **JOURNAL OF CLINICAL MICROBIOLOGY. 32(5):1341-2, 1994 May.**

Winsom C. DePaola LG. Thomas DL. Moss MW. Meiller J. Overholser C. Quinn TC. Prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) in dental school patients and dental practitioners in Maryland. **JOURNAL OF THE MARYLAND STATE DENTAL ASSOCIATION. 1993 Spring;35(1):17-9.**

Quinn TC. KlineRL. Moss MW. Livingston RA. Hutton N. Acid dissociation of immune complexes improve diagnostic utility of p24 antigen detection in perinatally acquired human immunodeficiency virus infection. **JOURNAL OF INFECTIOUS DISEASES. 167(5):1193-6, 1993 May.**

Bollinger RC Jr. Kline RL. Francis HL. Moss MW. Bartlett JG. Quinn TC. Acid dissociation increases the sensitivity of p24 antigen detection for the evaluation of antiviral therapy and disease progression in asymptomatic human immunodeficiency virus-infected persons. **JOURNAL OF INFECTIOUS DISEASES. 165(5):913-6, 1992 May.**

Abstracts

Johnson T. Morseman J. Ramnarain S. Zoha S. Moss M. Allnutt FCT. Direct fluorescent detection using PBXL dyes. **IBC CONFERENCE ASSAY MINIATURIZATION, SQUAW VALLEY, NEVADA FEBRURARY 1998.**

Kline R. Newhouse R. Granade T. Phillips S. Moss M. Quinn TC. Evaluation of the MicroTrak II HIV-1/HIV-2 Recombinant Antigen Enzyme Immunoassay. **94th GENERAL MEETING OF THE AMERICAN SOCIETY OF MICROBIOLOGY, LAS VEGAS, NEVADA MAY 1995.**

Kelen GD. Hexter DA. Kline R. Carella A. Moss M. Quinn TC. Dynamics of the HIV epidemic in an inner-city emergency department (ED): implications for ED based targeted screening. **IX INTERNATIONAL CONFERENCE ON AIDS, BERLIN, GERMANY JUNE 1993.**

Quinn TC. Kline R. Livingston R. Carella A. Moss M. Hutton N. An algorithm for the early diagnosis of perinatally acquired HIV-1 infection using the IgA immunoblot assay and the modified p24 antigen (Ag) assay. **IX INTERNATIONAL CONFERENCE ON AIDS, BERLIN, GERMANY JUNE 1993.**

Presentations

Comparison of absolute CD4+ lymphocyte counts by enzyme immunoassay(TRAx CD4 Test Kit) and flow cytometry. Oral presentation, Emerging Technologies Satellite Symposium- **IX INTERNATIONAL CONFERENCE ON AIDS, BERLIN, GERMANY JUNE 1993.**

Patents

Morseman JP. Moss MW. Allnutt FCT. High Fluorescent Intensity Crosslinked Allophycocyanin. **[pending]**

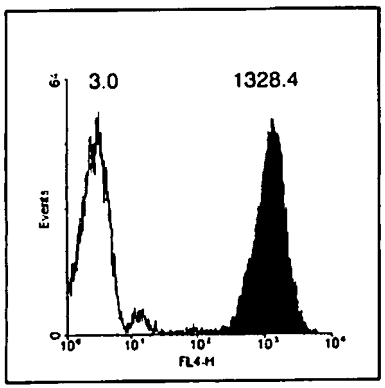
Morseman, JP. Moss MW. Ellis LA. Reelin Deficiency or Dysfunction and Methods Related Thereto. **[pending]**

Flow Cytometry

EL4 cells
biotin-anti-mouse Thy1.2

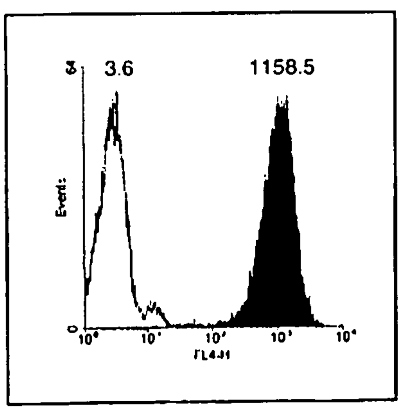
Mouse thymocytes labeled with biotinylated anti-mouse Thy1.2 and then stained with 4 µg/test Martek SureLight APC-Streptavidin or Molecular Probes XL-APC-Streptavidin. Analyzed using a 633nm red HeNe laser and a 670nm bandpass Filter.

XL-APC
4 ug/test



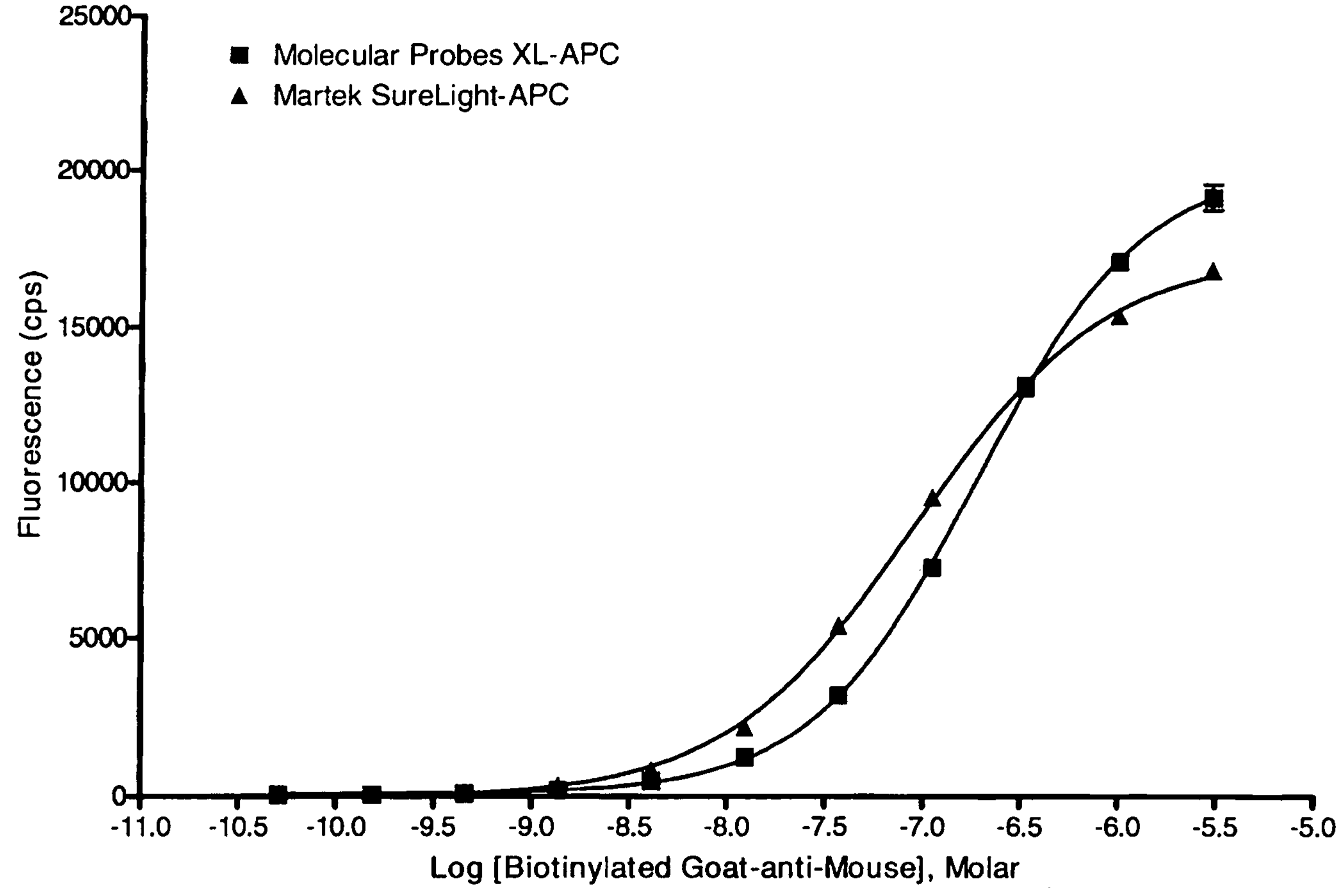
Molecular Probes XL-APC mean channel fluorescence= 1328.4

SL-APC
4 ug/test



Martek SureLight-APC mean channel fluorescence= 1158.5

Solid Phase Microplate Immunoassay



Black polystyrene microplate coated with mouse IgG, blocked, incubated with serial dilutions of biotinylated goat-anti-mouse, and detected with 10 μg/ml of Martek SureLight-APC Streptavidin or Molecular Probes XL-APC Streptavidin

Streptavidin and Fluorescent Conjugates of Streptavidin

Quick Facts

Storage upon receipt:

Unlabeled Streptavidin
- ≤–20°C
- Desiccate

Fluorescent-Dye Conjugates
- ≤–20°C
- Desiccate
- Protect from light

R-Phycoerythrin, B-Phycoerythrin, Allophycocyanin and Tandem Conjugates
- 2–6°C
- Do not freeze
- Protect from light

Introduction

Molecular Probes carries an extensive line of unlabeled and labeled streptavidin products (Table 1), including conjugates of R-phycoerythrin (R-PE), B-phycoerythrin (B-PE) and allophycocyanin (APC) (Table 2). The high affinity of avidin, an egg-white protein, for biotin was first exploited in histochemical applications in the mid-1970s.[1,2] Avidin and its bacterial counterpart, strept-avidin (from *Streptomyces avidinii*), have since become standard reagents for diverse detection schemes.[3] Streptavidin, a nonglycosylated 52,800-dalton protein, reportedly exhibits less nonspecific binding than avidin. However, streptavidin contains a tripeptide sequence Arg–Tyr–Asp (RYD) that apparently mimics the Arg–Gly–Asp (RGD) binding sequence of fibronectin, a component of the extracellular matrix that specifically promotes cellular adhesion.[4] This universal recognition sequence binds integrins and related cell-surface molecules.[5,6] Background problems sometimes associated with streptavidin may be attributable to this tripeptide.

In addition to the products listed in this product information sheet, Molecular Probes prepares conjugates of avidin and NeutrAvidin™ biotin-binding protein, fluorescent conjugates of many species-specific anti-IgG antibodies and conjugates of protein A and protein G. Visit our Web site (www.probes.com) for additional information.

Table 1. Unlabeled streptavidin and fluorescent-dye conjugates of streptavidin.

Label	Abs *	Em *	Catalog Number
Unlabeled streptavidin	NA	NA	S888
Fluorescent Dye Conjugates			
Alexa Fluor® 350	346	442	S11249
Marina Blue™	365	460	S11221
Cascade Yellow™	402	545	S11228
Alexa Fluor® 405	402	421	S32351
Pacific Blue™	410	455	S11222
Alexa Fluor® 430	434	539	S11237
Fluorescein	494	518	S8
Alexa Fluor® 488	495	519	S11223, S32354
Oregon Green® 488	496	524	S6368
DyeMer™ 488/605	502	609	S32385
DyeMer™ 488/615	502	615	S32386
DyeMer™ 488/630	502	630	S32387
Alexa Fluor® 500	503	525	S32352
Oregon Green® 514	511	530	S6369
Alexa Fluor® 514	518	540	S32353
Alexa Fluor® 532	530	554	S11224
Alexa Fluor® 555	555	565	S21381, S32355
Tetramethylrhodamine	555	580	S870
Alexa Fluor® 546	556	573	S11225
Rhodamine B	570	590	S871
Rhodamine Red™-X	570	590	S6366
Alexa Fluor® 568	578	603	S11226
Alexa Fluor® 594	590	617	S11227, S32356
Texas Red®	595	615	S872
Texas Red®-X	595	615	S6370
Alexa Fluor® 610	612	628	S32359
Alexa Fluor® 633	632	647	S21375
Alexa Fluor® 635	633	647	S32364
Alexa Fluor® 647	650	668	S21374, S32357
Alexa Fluor® 660	663	690	S21377
Alexa Fluor® 680	679	702	S21378, S32358
Alexa Fluor® 700	702	723	S21383
Alexa Fluor® 750	749	775	S21384

* Approximate absorption (Abs) and fluorescence emission (Em) maxima for conjugates, in nm. Complete spectra for most of these dyes are available at our Web site. † CMNB-caged fluorescein. NA = Not applicable.

Unlabeled Streptavidin

Streptavidin has four binding sites, permitting a number of techniques in which unlabeled streptavidin can be used to bridge two biotinylated reagents. This bridging method, which is commonly used to link a biotinylated probe to a biotinylated enzyme in enzyme-linked immunohistochemical applications, often eliminates the background problems that can occur when using direct streptavidin–enzyme conjugates.

Fluorescent Streptavidin Conjugates

Some of the simplest fluorescent detection schemes involving streptavidin entail applying a biotinylated probe to the desired sample and then detecting the bound probe with a fluorescently labeled streptavidin. Fluorescent conjugates of streptavidin are commonly used to localize antigens in cells and tissues [7,8] and to detect biomolecules in immunoassays and DNA hybridization techniques.[9–12]

R-PE, B-PE, APC and Tandem Conjugates of Streptavidin

Molecular Probes offers R-PE, B-PE and APC conjugates of streptavidin, as well as tandem conjugates of R-PE and APC labeled with long-wavelength Alexa Fluor dyes.

Our tandem conjugates comprise a donor phycobiliprotein, either R-PE or APC, conjugated to longer-wavelength light–emitting fluorescence acceptors. By the process of fluorescence resonance energy transfer (FRET), an energy transfer cascade is established wherein most of the light absorbed by the donor R-PE or APC results in fluorescence of the acceptor dye. This process can be quite efficient, resulting in almost total transfer of energy to the acceptor dye.

Our premium grade R-PE and APC conjugates of streptavidin have been highly purified to ensure that the products are: 1) predominantly 1:1 conjugates of R-PE or APC and streptavidin; 2) free of all unconjugated streptavidin; and 3) mostly free of unconjugated R-PE or APC. The premium grade R-PE and APC conjugates are a fractionation of our S-866 and S-868 products, respectively, intended especially for applications that benefit from additional purity. The purity of each lot is confirmed by analytical HPLC.

Molecular Probes' APC conjugates are prepared from chemically crosslinked APC to avoid dissociation of the molecule into subunits when highly diluted.[13]

Materials

Unlabeled Streptavidin

Unlabeled streptavidin (S888) is supplied as a lyophilized powder in a unit size of 5 mg. The specific activity is ~14 U/mg, where one unit is defined as the amount of protein required to bind 1 µg of biotin. Streptavidin is soluble to at least 10 mg/mL dissolved in phosphate-buffered saline (PBS) or other suitable buffer. This lyophilized product is stable for at least three years when stored desiccated at ≤–20°C. Reconstituted solutions are stable for approximately three months with the addition of sodium azide to a final concentration of 2 mM or thimerosal to 0.2 mM. For longer storage, divide solutions into aliquots and freeze at ≤–20°C. AVOID REPEATED FREEZING AND THAWING OF SOLUTIONS.

Table 2. R-PE, B-PE, APC and tandem conjugates of streptavidin.

Label	Abs *	Em *	Catalog Number
R-PE, B-PE and Tandem–R-PE Conjugates			
B-Phycoerythrin (B-PE)	546, 565 †	575	S32350
R-Phycoerythrin (R-PE)	496, 546, 565 †	578	S866, S21388 ‡
Alexa Fluor® 610–R-PE	496, 546, 565 †	630	S20982
Alexa Fluor® 647–R-PE	496, 546, 565 †	668	S20992
Alexa Fluor® 680–R-PE	496, 546, 565 †	702	S20985
Alexa Fluor® 750–R-PE	496, 546, 565 †	771	S32363
APC and Tandem-APC Conjugates			
Allophycocyanin (APC)	650	660	S868, S32362 ‡
Alexa Fluor® 680–APC	650	702	S21002
Alexa Fluor® 700–APC	650	723	S21005
Alexa Fluor® 750–APC	650	775	S21008

* Approximate absorption (Abs) and fluorescence emission (Em) maxima for conjugates, in nm. † Multiple absorption peaks. ‡ Premium grade.

Fluorescent Streptavidin Conjugates

Most of the fluorophore-labeled streptavidin products are supplied as lyophilized powder in 1 mg unit sizes. Conjugates with DyeMer™ fluorophores are supplied in 500 µg unit sizes. Certain conjugates are available as 2 mg/mL solutions in PBS, pH 7.2, containing 5 mM sodium azide, in 0.5 mL unit sizes. Peak absorption and emission wavelengths for these products are given in Table 1. The approximate degree of labeling of each conjugate is listed on the product's label. For the lyophilized products, solutions can be made by dissolving the powder in 0.5–1.0 mL of PBS or other suitable buffer. In the lyophilized powder form, the conjugates are stable for at least two years when stored at ≤–20°C. Reconstituted solutions are stable for approximately six months with the addition of sodium azide to a final concentration of 2 mM or thimerosal to 0.2 mM. Fluorescent streptavidin conjugates supplied in solution are also stable for approximately six months. For longer storage, divide solutions into aliquots and freeze at ≤–20°C. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING OF SOLUTIONS.

R-PE, B-PE, APC and Tandem Conjugates of Streptavidin

These products are stable for at least six months when stored undiluted at 2–6°C. DO NOT FREEZE R-PE, B-PE, APC OR TANDEM CONJUGATES. PROTECT FROM LIGHT.

Streptavidin–R-PE and Streptavidin–B-PE

The R-PE conjugates of streptavidin (S866, S21388) and the B-PE conjugate of streptavidin (S32350) are each shipped as a 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide, in a 1 mL unit size.

Streptavidin–APC

The allophycocyanin conjugates of streptavidin are each shipped as a 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide, in a 0.5 mL (S868) or 250 µL (S32362) unit size.

Tandem Conjugates of Streptavidin

The Alexa Fluor dye–R-PE and Alexa Fluor dye–APC tandem conjugates (Table 2) are shipped as 1 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, 2 mM EDTA, pH 7.5, containing 1% glycerol and 5 mM sodium azide, in unit sizes of 100 µL.

Applications

Streptavidin conjugates are used as secondary detection reagents in histochemical applications, flow cytometry,[14,15] blot analysis and immunoassays. These reagents can also be employed to localize biocytin, biocytin-X, biotin ethylenediamine and Alexa Fluor®, Cascade Blue® or lucifer yellow biocytins — derivatives of biotin that are used as neuroanatomical tracers.[16,17] The following are commonly used methods for employing streptavidin as a secondary detection reagent.

- **Direct Procedure:** A biotinylated primary probe such as an antibody, single-stranded nucleic acid probe or lectin is bound to tissues, cells or other surfaces. Excess protein is removed by washing, and detection is mediated by reagents such as our fluorescent streptavidins or our enzyme-conjugated streptavidins plus substrate.
- **Indirect Procedures:** A biotinylated antibody or oligonucleotide is used to probe a tissue, cell or other surface. This preparation is then treated with unlabeled streptavidin. Excess reagents are removed by washing, and detection is mediated by a biotinylated detection reagent such as fluorescein biotin (B1370), biotinylated R-PE (P811), biotinylated FluoSpheres® microspheres or a biotinylated enzyme (P917) plus a substrate. Alternatively, an unlabeled primary antibody is bound to a cell followed by a biotinylated species-specific secondary antibody. After washing, the complex is detected by the direct or indirect procedures described above.

It is a good practice to centrifuge protein conjugate solutions briefly in a microcentrifuge before use; only the supernatant should then be used for the experiment. This step will eliminate any protein aggregates that may have formed in solution, thereby reducing nonspecific background staining. Because staining protocols vary with the application, appropriate dilutions of conjugates should be determined empirically. For fluorescent dye conjugates of streptavidin, including R-PE, B-PE, APC and tandem conjugates of streptavidin, a final concentration of 1–10 µg/mL is usually satisfactory for most histochemical applications.

References

1. Proc Natl Acad Sci USA 71, 3537 (1974); **2.** Biochim Biophys Acta 264, 165 (1972); **3.** *"Avidin-Biotin Technology,"* M. Wilchek and E. Bayer, Eds., Meth Enzymol 184 (complete volume); **4.** Biochem Biophys Res Comm 170, 1236 (1990); **5.** Eur J Cell Biol 60, 1 (1993); **6.** Eur J Cell Biol 58, 271 (1992); **7.** J Cell Biol 111, 1183 (1990); **8.** Physiol Plantarum 79, 231 (1990); **9.** Cytometry 11, 126 (1990); **10.** Proc Natl Acad Sci USA 87, 6223 (1990); **11.** Science 249, 928 (1990); **12.** Anal Biochem 171, 1 (1988); **13.** Cytometry 8, 91 (1987); **14.** J Microbial Methods 12, 1 (1990); **15.** Biochemistry 16, 5150 (1977); **16.** J Neurosci 10, 3421 (1990); **17.** Brain Res 4~~97, 361 (1989)~~.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
S888	streptavidin	5 mg
S11249	streptavidin, Alexa Fluor® 350 conjugate	1 mg
S32351	streptavidin, Alexa Fluor® 405 conjugate	1 mg
S11237	streptavidin, Alexa Fluor® 430 conjugate	1 mg
S11223	streptavidin, Alexa Fluor® 488 conjugate	1 mg
S32354	streptavidin, Alexa Fluor® 488 conjugate *2 mg/mL*	0.5 mL
S32352	streptavidin, Alexa Fluor® 500 conjugate	1 mg
S32353	streptavidin, Alexa Fluor® 514 conjugate	1 mg
S11224	streptavidin, Alexa Fluor® 532 conjugate	1 mg
S11225	streptavidin, Alexa Fluor® 546 conjugate	1 mg
S21381	streptavidin, Alexa Fluor® 555 conjugate	1 mg
S32355	streptavidin, Alexa Fluor® 555 conjugate *2 mg/mL*	0.5 mL
S11226	streptavidin, Alexa Fluor® 568 conjugate	1 mg
S11227	streptavidin, Alexa Fluor® 594 conjugate	1 mg
S32356	streptavidin, Alexa Fluor® 594 conjugate *2 mg/mL*	0.5 mL
S32359	streptavidin, Alexa Fluor® 610 conjugate	1 mg
S20982	streptavidin, Alexa Fluor® 610–R-phycoerythrin conjugate (Alexa Fluor® 610–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL
S21375	streptavidin, Alexa Fluor® 633 conjugate	1 mg
S32364	streptavidin, Alexa Fluor® 635 conjugate	1 mg
S21374	streptavidin, Alexa Fluor® 647 conjugate	1 mg
S32357	streptavidin, Alexa Fluor® 647 conjugate *2 mg/mL*	0.5 mL
S20992	streptavidin, Alexa Fluor® 647–R-phycoerythrin conjugate (Alexa Fluor® 647–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL
S21377	streptavidin, Alexa Fluor® 660 conjugate	1 mg
S21378	streptavidin, Alexa Fluor® 680 conjugate	1 mg
S32358	streptavidin, Alexa Fluor® 680 conjugate *2 mg/mL*	0.5 mL
S21002	streptavidin, Alexa Fluor® 680–allophycocyanin conjugate (Alexa Fluor® 680–allophycocyanin streptavidin) *1 mg/mL*	100 µL
S20985	streptavidin, Alexa Fluor® 680–R-phycoerythrin conjugate (Alexa Fluor® 680–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL